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A SEARCH FOR EXTRATERRESTRIAL EUKARYOTES: BIOLOGICAL AND PLANETARY SCIENCE ASPECTS (*)

J. Chela-Flores (+)

International Centre for Theoretical Physics, 34100 Trieste, Italy ,and

Dublin Institute for Advanced Studies,10, Burlington Road, Dublin 4, Ireland.

Abstract. One pressing question in Bioastronomy is to attempt to devise assays in the search for extraterrestrial eukaryotes (SETE). In the positive case of encountering a fossil of a certain microorganism, it is not yet clear how to identify it in an unequivocal manner, even if found arrested and preserved in the midst of biological activity, as in some known Archean sediments on Earth. On the other hand, for living microorganisms the present approach may have some advantages over the straightforward probe of the morphological features of the putative eukaryote, as we argue that we are not constrained to base identification on morphological properties, such as the presence of organelles, which are known to be missing in some higher taxa. The present work suggests one possible assay to detect eukaryotes, namely, the search for cellular division with a delay in replication of heterochromatic chromosome segments; we also discuss the biological and astronomical implications of a SETE program. Further, 'extremophiles' may inhabit deep in the silicate crust of terrestrial planets which may have been deposited with the original sediment and survived over geologic time. Comparative planetology suggests that there are common ingredients in the nature of the landscapes of the terrestrial planets, particularly gradation. Hence, a SETE program should look for indicators of the first steps towards eukaryogenesis, for instance deep in the Martian crust. Additional ingredients in the common landscape of the rocky planets, or satellites, may be volcanism and tectonics. This has led to the belief that hot springs may be present at the bottom of Europa's great ocean. Hence, eukaryotes should also be searched for in this aquatic environment. Our current work emphasising gene silencing, may suggest how to decide whether extraterrestrial microbiota may have taken the first steps towards eukaryogenesis, the process which introduced the basic cellular plan of those Earth-bound organisms that have been raised to the level of intelligent beings.

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(+) Also Instituto Internacional de Estudios Avanzados, Caracas 1015A, Venezuela.

1. Introduction: the search for early terrestrial eukaryotes

There is general agreement concerning certain aspects of the first appearance in the fossil record of a nucleated cell, or eukaryote. This event may have occurred during the Proterozoic eon in an environment that was dominated exclusively by prokaryotes, which are single-celled organisms without an inner membrane enveloping their genomes. This phenomenon is referred to below as 'eukaryogenesis'. It was possibly the most significant evolutionary step of the Proterozoic eon, since it provided the cellular theme on which intelligence was to develop in the subsequent Phanerozoic eon. In other words, the earliest complete eukaryote with organelles, nucleus, and cell-wall was a morphologically simpler organism than most protists, or cells of contemporary metazoans and metaphytes. These early, yet complex cells may have resembled primitive algae.

This consensus view raises some problems, since such an early eukaryote is not essentially different in size, shape and mode of cell division (binary fission) from a coccoid cyanobacterium, a prokaryote that is known to have also been abundant throughout the Proterozoic, and whose first appearance occurred during the preceding eon, the Archean (Schopf, 1993).

Rather than addressing the significant question of the origin of the eukaryotic cell from their prokaryotic ancestors (De Duve, 1995; Margulis, 1993), in this paper we choose to address two relatively simpler problems:

(I) What are the criteria by means of which a primitive eukaryote may be distinguished from a prokaryote?, and

(II) Does it make sense to search for life in the Solar System and beyond and, if so, what is the relevance of the distinction between eukaryotes and prokaryotes?

Yet, even these restricted questions are still of considerable difficulty, in spite of substantial earlier work. On the one hand, we have Archean and Proterozoic micropaleontology, an extensive field of research, which matured in the 1970s and has established itself since then (Schopf and Klein, 1992). On the other hand, we have the relevant work on a possible oxygen-rich state, early in the evolution of the atmosphere in Mars (McKay, 1996).

Some comments are needed in order to clarify the above two questions. First of all, regarding question (I), part of the difficulty lies in the fact that micropaleontological signatures for early-developing eukaryotes are cell size and shape. In terms of these two parameters, attempts are normally made to distinguish amongst a wide distribution of acritarchs in fossiliferous rocks between fossils of prokaryotes and those of eukaryotes. In fact, it is precisely because of this situation that the actual time of eukaryogenesis still remains a mystery. To sum up, all three domains of microorganisms, Bacteria, Archea and Eukarya (Kandler, 1995) may be similar with respect to parameters, such as size, shape and mode of cellular division. (In this context, in the case of Eukarya, we have restricted ourselves exclusively to the deepest branches of the phylogenetic tree, all of which are represented by single-celled eukaryotes, or 'protists'.)

Secondly, concerning question (II), the underlying difficulty is to decide whether life may be widespread in the universe or not. A straightforward interpretation of darwinian evolution includes the underlying mutations that perturb the faithfulness of DNA replication, as well as environmental challenges that may influence evolution, thus allowing natural selection to play its central role. Both of these factors imply a low probability for the emergence of life in the universe (Monod, 1971; Mayr, 1995). Yet, we may still argue that the underlying contingency that we have just emphasised (i.e., the above-mentioned two darwinian factors that imply that the shaping of the present may result from unpredictable antecedent states) is, nevertheless, highly constrained in the case of the eukaryotic genome. Recently, the general question of 'constrained contingency' has been discussed (De Duve, 1995). We shall return shortly to this topic.

In spite of the difficulties raised by question (I), some options remain open to the micropaleontologist, namely the ultrastructure of the microorganism and the elemental composition of the preserved cell walls. The second of these questions will be considered later.

The main thesis we wish to develop and defend here arises from the experience that has been gathered from the study of the lower branches of the phylogenetic tree of Domain Eukarya. In fact, not only are size and shape ambiguous signatures for early-evolving eukaryotes, but moreover a more serious impediment is that few ultrastructure properties are unmistakable signs of eukaryoticity.

Questions (I) and (II) have induced us to focus our attention on the eukaryotic genome. This approach may help us to identify a hallmark of eukaryogenesis, namely heterochromatin (i.e., the maintenance of the condensed state during interphase in the cell cycle). As a means of gene regulation heterochromatin is an aspect of the genome of the eukaryotes which deserves special consideration. We propose to focus attention on this aspect of chromosomes for several reasons, most prominent amongst which is the restriction of randomness in the evolution of eukaryotes.

Indeed, chance processes may induce rearrangements of this form of chromatin (i.e., 'constitutive' heterochromatin), that may still preserve general chromosome organization. This may provide a concrete example of a 'constraint on contingency', a concept that has a far-reaching consequence on the central question of our work, namely the ubiquity of life in the universe.

In the companion work (Chela-Flores, 1996b) we shall illustrate, with a specific example, the above-mentioned constraint on contingency which is imposed by the heterochromatic state of chromatin. The example will be extracted from two genera of the same dicot family (Solanaceae) and will serve to illustrate the fact that substantial rearrangements of heterochromatin do not necessarily induce radical phenotypic changes in some metaphytes. The main point worth anticipating here is that this tendency of the genome, namely, counteracting the effect of chance mutations by preserving general chromosme organization, demonstrates a certain 'genome plasticity'. We shall discuss more fully this concept in our subsequent work.

The contribution of endosymbiosis to eukaryogenesis

We have considered recently the main problems concerning eukaryogenesis (Chela-Flores, 1994a): Eukaryotes have their DNA linked in chromatin, which lies essentially in its nucleus, but it may also be constrained inside organelles in the cytoplasm such as mitochondria, chloroplasts, and nucleomorphs. Some further organelles do not contain DNA; for instance, there are certain particulate entities, somewhat smaller than the mitochondrion, such as the perioxisomes which are ubiquitious amongst the eukaryotes;

and the hydrogenosomes, which are obseved in certain human parasitic anaerobic protists, the trichomonads.

However, some protists are mitochondrion-less eukaryotes. There is even a whole Eukarya kingdom of amitochondrial protists, the microsporidia (Cavalier-Smith, 1987a). Besides, in two out of three kingdoms of Domain Eukarya (i.e., Animalia and Fungi) chloroplasts are absent. The origin of organelles in the eukaryotes is to be found, according to the Serial Endosymbiosis Hypothesis (Margulis, 1993), in separately evolved organisms. The effect of this evolutionary force is particularly clear in the case of the nucleomorph, an organelle which has been observed in certain cryptomonad flagellates (Douglas *et al.*, 1991).

In fact, symbiosis implies that the prokaryote ancestral to the mitochondrion may have been taken up by a chloroplast-free amoeboid protoeukaryote (a phenomenon which is referred to as 'phagocytosis'). Eventually the symbiont may have lost autonomy, possibly by horizontal gene transfer between the protomitochondrion and the host's nucleus. These events were factors that would have led to the evolution of a single-celled organism that had already integrated the metabolism of the partners in symbiosis.

Further support for symbiosis is found in the fact that two of the eukaryotic organelles, the mitochondrion and the chloroplast, have their own specific mechanisms of translation; in fact, genetic codes which deviate from the standard code are known for both the mitochondrion as well as for the chloroplast. However, the origin of the nucleus does not seem to be explained by symbiosis. These factors, as well as the several examples we have just discussed, demonstrate that not only is the actual time of eukaryogenesis unknown, but the nature of the earliest eukaryote still remains an open problem.

On the other hand, some insights on the transition prokaryote-eukaryote may be obtained from certain groups of primitive eukaryotic organisms that have been studied in great detail. One such taxon is the family Cyanidiophyceae. These organisms are rhodophytes, commonly known as red algae (Seckbach, 1994a). It has been argued that these acido-thermophilic algae may constitute a transitional organism between cyanobacteria and red algae (Seckbach, 1994b). It has been shown that a specific species of those primitive algae may

thrive in a simulated atmosphere of carbon dioxide. For this reason it is clearly useful to know in which atmospheres extreme organisms may survive.

Solar System exploration and the evolution of microorganisms

We know that several Solar-System planets and satellites have atmospheres. Yet, none of them are similar to our own. For example, four large planetary satellites in the outer Solar System are known to have atmospheres (Hall *et al.*, 1995): the Jupiter satellites Europa and Io; the large Saturnian moon Titan, and Neptune's satellite Triton. Both terrestrial planets, Venus and Mars have CO_2 atmospheres.

The possibility of extending the biosphere deep into the silicate crust in another terrestrial planet (Mars) deserves special attention (Hartman and McKay, 1995); this will be considered below. The present status of the search for life on Mars consists essentially of the Viking results, which are reviewed in Table 1.

TABLE 1: The question of life on Mars (based on Soffen, 1976).

Experiment	***Results***
Test for signs of photosynthesis or chemosynthesis induced by samples from the soil	Small incorporation of CO/CO_2 into organics
Measurement of any gaseous products from a soil sample	Initial rapid release of O_2; slow release of CO_2 , N_2
Search for the release of radioactive gas when the soil sample was exposed to a radioactive organic nutrient solution	Initial rapid release of labelled gas, followed up by slow release

However, UV radiation in the absence of a large fraction of oxygen in the Martian atmosphere (i.e., an ozone layer) prevents the possibility of life on the surface of that planet since, at least on the planetary surface, there are no evident UV defense mechanisms; but one possibility is represented by life underground, as a layer of permafrost could serve as the necessary UV defense mechanism (Farmer and Doms, 1979). This question seems pertinent to exobiology, since we cannot exclude at present that the organisms that have been found to inhabit deep in the silicate crust of the Earth may have been deposited with the original sediment.

There are several additional reasons why planetary science may help in some of the most basic questions of the biology of eukaryotes. Already considerable expertise has been gained over the last 30 years from radioastronomy in the central problem of exobiology, the search for extraterrestrial intelligence, SETI (Drake, 1996). A consensus view that has emerged from this effort is that life must be a widespread phenomenon in the universe. Some support for this expectation is provided by the above-mentioned constraints on contingency that severely limit the apparently unlimited possibilities that chance may impose on the evolutionary processes. Our concept of genome plasticity militates in favour of this possibility, particularly in the case of the eukaryotes.

On the other hand, more restricted searches for signatures of life, other than radio signals from advanced civilizations, are currently being proposed. By fixing attention on the homochirality of the biomolecules, the search for extraterrestrial homochirality (SETH) has been proposed (MacDermott and Tranter, 1995; MacDermott *et al.*, 1996). Some insights into life's origins in the Solar System may be gained from SETH without imposing an unbearable payload in forthcoming space missions. Moreover, between the two extremes of the most basic (SETH) and most advanced manifestations of life (SETI), lies the above-mentioned fundamental search for extraterrestrial eukaryotes (SETE).

The relevance of a SETE program cannot be overemphasised, since it is the evolution of the eukaryotic cell that sets living organisms onto the path of multicellularity. This fundamental capability which eukaryotes acquired in the Neoproterozoic (Ediacara faunas, diploblastic grades) led in the early Phanerozoic to

triploblastic phyla (Chengjlian fauna, Lower Cambrian, some 535 million years, Myr, before the present) and eventually to intelligent beings in the Cenozoic era (66 Myr to the present).

At present we are beginning to realise with more than one example of extra Solar System planets that favourable environments for life's origins, comparable to the one that has been provided by the Earth, may also occur in our own galaxy, even in planets around stars in the vicinity of the Sun (Schneider, 1996).

The search for extraterrestrial eukaryotes

The question of the possible existence of microorganisms in the Solar System has been raised in the past, in spite of the Viking results, which we have already referred to in Table 1 (McKay and Stoker, 1989). The special case of the possible occurrence of eukaryotes during an earlier epoch has been raised during the 1995 Trieste Conference (McKay, 1996).

Thus, we discuss here two possible Solar-System environments in which a SETE program may be relevant, Mars and the Jupiter satellite Europa, basing our discussion in the case of the Jovian moon on an extension of some arguments that Oro presented at the same Trieste Conference (Oro, 1996).

Mars. Our nearest-neighbour planet is a candidate for having supported life in the past. We cannot exclude its presence in some isolated environments. Life may have evolved during an early clement period that may have occurred in the Noachian epoch, or early Hesperian in Mars stratigraphy, according to the terminology introduced by Tanaka (cf. Table 1 in Sleep, 1994 for references to the original literature). Possible candidates for sites in which life may have evolved are located in the Tharsis region in the northern hemisphere, where volcanic activity has taken place since, by analogy with the Earth, the heat from underground magma may have produced hot springs, which are known to be possible sources of hyperthermophilic microorganisms. Knowledge of these possible locations raises the question whether life may have survived till the present confined to regions where pockets of liquid water may occur.

These considerations lead us to the conclusion that at present we cannot exclude the possible presence of either single-cell eukaryotes, or even multicellular organisms from the Martian fossil record (McKay, 1996).

Europa. A second candidate for a SETE program is the Jovian satellite Europa, as a large proportion of the spectroscopically detectable material on its surface is water (Oro, 1996; Horneck, 1995). According to the results on the Jupiter system obtained by the Voyager 1 mission, Europa is covered by a layer of ice, under which there may be an ocean of water, whose temperature is 4 ^{o}C. From the similarity of the processes that gave rise to the solid bodies of the Solar System, we may expect that hot springs may lie at the bottom of the ocean. Oro's main thesis is that, as Jupiter's primordial nebula must have contained many organic compounds, then possibly, organisms similar to thermophilic archaebacteria could exist at the bottom of Europa's ocean.

However, given the incomplete understanding of the evolution of early life on Earth, at present we should extend the Oro argument in order to include eukaryotes as well. In this respect we may add that up to the present the divergence into the three domains, arising from the evolution of the progenote, is not well understood. Indeed, plate tectonics has obliterated fossils of early organisms from the crust of the Earth, which is the only record available to us.

Several additional factors arising from current experience with archaebacteria and eukaryotes may contribute to clarify the case for not excluding eukaryotes from the microorganisms to be searched in new environments.

(i) The triple junction between the branches of the three main domains was assumed to be close to the progenote. Later, the root of the tree has been assumed to lie along the eubacterial branch (Cavalier-Smith, 1987a,b). This may imply that a critical step in the diversification of single-cell organisms may have been the loss of the ability to manufacture a cell-wall biomolecule (peptidoglycan murein). Without the constraint that this biomolecule imposes on cell shape, both Archaea and Eukarya have been able to diversify beyond the Domain Bacteria.

(ii) In spite of the fact that eukaryotes and archaebacteria have both lost the ability to synthesise the molecule peptidoglycan murein, we can nevertheless distinguish between them, as all eukaryotes have ester lipids in their membranes, whereas archaebacteria have ether lipids.

(iii) Earth-bound eukaryotes are not extremophiles, but their diversification may share a common thread with archeabacteria. Eukaryotes, in spite of not being able to exploit fully all the environments available to the extremophiles, nevertheless, may live in anoxic conditions up to temperatures well above room temperature (57 ^{o}C). Some eukaryotes may even tolerate very acidic growing media of pH 2-4, as it has been previously shown in the case of a primitive rhodophyte (Seckbach, 1972; 1994a,b, 1996).

To sum up, although it may be unlikely for eukaryotes to have evolved in the environmental conditions currently hypothesised for Europa, at present we lack experience with darwinian evolution in extraterrestrial conditions. This induces us to think that present and future efforts should not be confined to the possibility of designing equipment capable of recognising exclusively archaebacteria in a future mission to Europa. On the contrary, tests for recognising eukaryotes should also be envisaged.

A hallmark for Eukarya: concepts from molecular genetics

In order to reinforce the above proposals, in the present section we describe, in an abbreviated form, the necessary concepts from molecular biology and genetics. Our work supports the persuasive arguments of McKay and co-workers, which are complementary to our own approach, as they have been considering arguments on the possibility of the existence of extraterrestrial eukaryotes from the point of view of geophysics.

During interphase in the cell cycle a solenoidal arrangement constitutes the most abundant form of chromatin with packaging density (pd) of the order of 10^2. (We recall that pd is defined as the quotient of the length of DNA in the fully extended state divided by the length of DNA in the folded state.) However, at later stages in the cell cycle this structure serves as the basis for further folding, ending

up at the highest degree of folding observed at the metaphase chromosome, whose value for the pd may reach 10^4.

In view of the basic role played by all the stages of the hierarchy, it is of some interest to find eventually a formalism by means of which we can anticipate the rather regular manner in which DNA folds in chromatin. This problem is still not understood and is of considerable difficulty (Chela-Flores, 1994b). However, our present lack of understanding of the DNA folding problem does not prevent us from attempting to make use of the folded state with its maximum value of pd (i.e., heterochromatin) as a hallmark of eukaryogenesis. What is required from the point of view of exobiology is to identify possible tests for eukaryoticity, amongst the various properties of chromatin compaction in the processes underlying the structure and function of chromosomes.

In this context, the problem of DNA replication may point the way to proceed, since DNA polymerases initiate their catalytic action in numerous replication origins. In such loci we may find a hint of a typical eukaryotic mechanism, the recognition of which is our aim in our efforts to single out significant eukaryotic hallmarks form the point of view of genetics.

One possible hint may be obtained from the temporal order for the initiation of origins. This process is controlled at the level of chromatin structure. Such control, which is evident in the origins of DNA replication, is most prominent though in the regulation of cell division. Indeed, it is during cell division that a clearer manifestation of chromatin structure becomes apparent. For instance, genes on heterochromatin characteristically go through DNA replication late in the cell cycle (Lima-De-Faria and Jaworska, 1968). This property is an obvious candidate to be used in the design of tests of eukaryoticity in future planetary exploration.

The proposed hallmark of eukaryogenesis has the advantage of being part of the mechanism of cell division. It should not escape our attention that the mode of cell division itself is one of the clearest signatures of single-celled organisms, which may be evident even in fossils of microorganisms that have been caught in the midst of biological activity. (In the next section we shall return to the possible fossil evidence of populations of microorganisms that may have preserved their mode of cell division.)

Discussion and conclusions

We have attempted to define some aspects of the problem of the origin and evolution of the first nucleated cell. We have emphasised the role of DNA packaging in a gene-centred approach to eukaryogenesis. The underlying physical mechanism is a two-stage process, first DNA bending round the nucleosome core and secondly, DNA folding through all the stages leading up to the heterochromatic metaphase chromosome.

One pressing question in exobiology is whether, by adopting an approach such as the present one (a gene-centred approach), rather than the traditional cytological criterion for a eukaryotic cell, we may attempt to devise assays for investigating whether what we encounter in a search for life by, for instance a Mars lander, is a prokaryotic or a eukaryotic organism.

In the positive case of identifying a fossil of a certain microorganism, it is not yet clear how to identify in an unequivocal manner whether there are traces of heterochromatin in the given fossil. In such a case, we may have to rely more on cell-membrane biomarkers.

On the other hand, if *living microorganisms* were to be encountered in a future space mission, the present approach may have advantages over straightforwardly probing the morphological features of the putative eukaryote, as we do not base its identification on morphological properties, such as the presence of organelles. The present work suggests one possibility for such an assay: one may search for cellular replication and investigate whether there is a delay in replication of chromosome segments, which would characterize them as being heterochromatic, according to the above-mentioned experiments of Lima-de-Faria and co-workers.

It should be noticed that the search for a membrane-bounded set of chromosomes (in a single-celled organism that might be encountered) evidently does not answer the question, as there are prokaryotes, such as *Gemmata oscuriglobus* that have a membrane-bounded nucleoid (Fuerst and Webb, 1991).

As stated previously, we cannot exclude at present organisms that may inhabit deep in the silicate crust of the rocky planets. The case of the Earth suggests this possibility. Such 'extremophiles' may

have been deposited with the original sediment and survived over geologic time (Parkes and Maxwell, 1993). Hence, if such organisms are alive deep in the Martian crust, then assays should be formulated to acertain whether the first steps towards eukaryogenesis have been taken (as suggested above). In this context we should recall the constraints that are implied by the eventual knowledge of the oxidation state of the Martian surface. With the assumption that life has been supported beneath the Martian surface, some models have been developed in which biological sources could have extended the depth of oxidation deep underground. The exact depth being hundreds of meters if life persisted up to a billion years (Hartmann and McKay, 1995).

Comparative planetology suggests that there are common ingredients in the nature of the landscapes of the terrestrial planets, particularly gradation (alteration due to weathering, erosion and sedimentation). Hence, a SETE program should look for indicators of the first steps towards eukaryogenesis, for instance deep in the Martian crust. Further, ingredients in the formation of a common landscape of the rocky planets or satellites may be volcanism and tectonics. This follows from simultaneous consideration from several planets and the Moon. These common features may imply that the eventual presence of hot springs at the bottom of Europa's great ocean cannot be ruled out. We have defended above the Oro thesis in a wider sense, so as to include eukaryotes, which should consequently be searched for in this acquatic environment, provided the considerable underlying technical problems related with oceanography could be solved.

In solar system planets, or in the several approximately Jupiter-sized planets now known to be around nearby stars, in which detailed atmospheric evolution remains unknown, the search for life should not be constrained to questions related only to microorganisms that are *a priori* assumed to resemble either bacteria or archaebacteria. Our current work emphasising gene silencing (Chela-Flores, 1994a; 1996a), may suggest how to decide whether a given microorganism in extraterrestrial environments has taken the first steps towards eukaryogenesis.

There is an additional related point worth considering in the context of cell division in eukaryotes. In the future, planetary rovers

may encounter a situation analogous to some work on Archean fossils: A population of microstructures may fossilize consisting of microorganisms arrested and preserved in the midst of biological activity. Such possibility would represent an opportunity for gaining some insights into the level of evolution of the microorganisms that yielded the given fossils, but it is not clear whether a clear signature of eukaryoticity could be inferred. It is precisely this situation that was encountered in a population of organic walled microstructures from the Swaziland System in South Africa (Knoll and Barghoorn, 1977).

Our emphasis, in the work discussed here of identifying a genetic hallmark of eukaryogenesis, rather than morphological features of the cell may suggest ways of linking eukaryogenesis with cell division. One such possibility is to consider more evident ultrastructure of fossilized membrane molecules of cell walls, such as steranes; this is an important group of abundantly distributed biomarkers which have been characterised from oils and sediments, and whose parental material (tetracyclic steroids) is almost exclusively found in eukaryotes (Chadha, 1995).

We expect that further work along these lines may ultimately lead to the preparation of feasible, useful experiments for *in situ* exploration of planetary or satellite environments for the presence of eukaryotes, or their fossils.

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